

## Following TRAIL's path in the immune system

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### Summary

The members of the tumour necrosis factor (TNF) superfamily of cytokines play important roles in the regulation of various immune-cell functions. Likewise, induction of cell death by apoptosis is indispensable for the normal functioning of the immune system. There are two major pathways of apoptosis induction. The intrinsic, or mitochondrial, pathway is regulated by the activation and interaction of members of the Bcl-2 family. The extrinsic, or death receptor, pathway is triggered by certain TNF family members when they engage their respective cognate receptors on the surface of the target cell. Hence, cell-to-cell-mediated death signals are induced by activation of these death receptor–ligand systems. Besides TNF itself and the CD95 (Fas/APO-1) ligand (FasL/Apo1L), the TNF-related apoptosis-inducing ligand (TRAIL/Apo2L) belongs to the sub-family of ligands that is responsible for extrinsic induction of cell death. Depending on their status of stimulation, TRAIL can be expressed by various cells of the immune system, amongst them natural killer (NK) cells, T cells, natural killer T cells (NKT cells), dendritic cells and macrophages. TRAIL has been implicated in immunosuppressive, immunoregulatory and immune-effector functions. With respect to pathological challenges, TRAIL and its receptors have been shown to play important roles in the immune response to viral infections and in immune surveillance of tumours and metastases. In this review we summarize the current knowledge on the role of TRAIL and its receptors in the immune system and, based on this, we discuss future directions of research into the diverse functions of this fascinating receptor–ligand system.

**Keywords:** apoptosis; immune system; tumour necrosis factor-related apoptosis-inducing ligand (TRAIL); tumour immunity

### Introduction

Various members of the tumour necrosis factor (TNF) and the TNF receptor (TNF-R) superfamily have been shown to exert important functions in the immune system. One of these functions is the induction of apoptosis, which is mediated by certain members of the TNF and TNF-R families known as death ligands and receptors, respectively. Apoptosis is essential for the proper function of the immune system. It serves different purposes, amongst them tasks as divergent as the removal of auto-reactive T and B cells, the killing of infected cells by cytotoxic lymphocytes and the down-modulation of immune responses after an infection has been successfully dealt

with.<sup>1</sup> Dysregulation of apoptosis in cells of the immune system can have severe consequences, which may result in diseases, including cancer and autoimmunity.

The TNF-related apoptosis-inducing ligand (TRAIL) and its five cellular receptors constitute one of the three death-receptor/ligand systems that have been shown to regulate intercellular apoptotic responses in the immune system. In different systems of antigenic or tumour challenge, the TRAIL/TRAIL receptor system was shown to have immunosuppressive, immunoregulatory, proviral or antiviral, and tumour immunosurveillance functions.<sup>2,3</sup>

This review summarizes our current understanding of the role of the TRAIL system in immunology and aims to point out future directions of research. Before we turn

our attention to the functions of the TRAIL system in immunology we will, however, briefly summarize the molecular make-up of this receptor–ligand system and show how it is thought to initiate the intracellular signalling cascade of apoptosis.

### TRAIL and its receptors

TRAIL can bind two apoptosis-inducing receptors – TRAIL-R1 (DR4) and TRAIL-R2 (DR5) – and two additional cell-bound receptors incapable of transmitting an apoptotic signal – TRAIL-R3 (LIT, DcR1) and TRAIL-R4 (TRUNDD, DcR2) – sometimes called decoy receptors. Finally, a soluble receptor called osteoprotegerin (OPG) is also capable of binding TRAIL.

The initial step of apoptosis induction by TRAIL is the binding of the ligand to TRAIL-R1 or TRAIL-R2. Thereby the receptors are trimerised and the death-inducing signalling complex (DISC) is assembled.<sup>4</sup> The adaptor molecule, Fas-associated death domain (FADD), translocates to the DISC where it interacts with the intracellular death domain (DD) of the receptors. Via its second functional domain, the death effector domain (DED), FADD recruits procaspases 8 and 10 to the DISC where they are autocatalytically activated. This activation marks the start of a caspase-dependent signalling cascade. Full activation of effector caspases leads to cleavage of target proteins, fragmentation of DNA and, ultimately, to cell death.

In type I cells, the DISC-induced caspase cascade is sufficient for the activation of effector caspases and hence the induction of apoptosis. By contrast, type II cells depend on the activation of the mitochondrial amplification loop, also called the intrinsic, or Bcl-2-controlled, apoptosis pathway. The death receptor-mediated extrinsic pathway and the mitochondrial intrinsic apoptosis pathway are connected via the BH3-only protein, Bid. Upon DISC formation, Bid is cleaved to truncated Bid (tBid) by caspase-8 or caspase-10. tBid translocates to the mitochondria, leading to the activation of Bax and Bak and, consequently, to the release of cytochrome *c* and other pro-apoptotic proteins from the mitochondria.<sup>5</sup> Cytochrome *c*, together with dATP, apoptotic protease activating factor 1 (Apaf-1) and caspase-9, forms the apoptosome, which serves as an activation platform for the initiator caspase-9 that in turn activates downstream effector caspases. Additionally, second mitochondria derived activator of caspases/direct IAP binding protein with low pI (Smac/DIABLO), which is also released from the mitochondria during apoptosis, counteracts the function of X-linked inhibitor of apoptosis protein (XIAP), thereby allowing for full activation of caspases 3, 7 and 9.<sup>6</sup> A schematic overview of the TRAIL apoptosis pathway is shown in Fig. 1.

In contrast to the human TRAIL/TRAIL-R system, mice only possess one apoptosis-inducing receptor, called murine TRAIL-R (MK, mDR5), which is equally homologous

to human TRAIL-R1 and TRAIL-R2.<sup>7</sup> The other murine receptors, mDcR1, mDcR2L and the splice variant mDcR2S, share a clustered locus.<sup>8</sup> However, functionally, they have not been studied in any particular detail.

### Expression and function of TRAIL in the innate and adaptive immune systems

The first hint at understanding the function of TRAIL in the immune system came when it was discovered that TRAIL is expressed on a variety of cells of the innate and adaptive immune systems. Yet, the expression of TRAIL was found to depend on the stimulation status. TRAIL is up-regulated on monocytes and macrophages after stimulation with lipopolysaccharide (LPS) and interferon- $\beta$  (IFN- $\beta$ ).<sup>9,10</sup> Interferon- $\gamma$  (IFN- $\gamma$ ), in turn, can induce the expression of TRAIL on the surface of monocytes, dendritic cells (DCs) and natural killer (NK) cells.<sup>11,12</sup> Surface-bound TRAIL is one of the effector mechanisms of NK cells, as only combined neutralization of TRAIL, CD95L and perforin can block NK cell-mediated killing of tumour cell lines *in vitro*.<sup>13</sup> These results were confirmed in an *in vivo* model, where TRAIL plays a critical role in NK cell-mediated and IFN- $\gamma$ -dependent suppression of tumour cell growth.<sup>14</sup> Furthermore, it was demonstrated that IFN- $\gamma$  induces TRAIL on NK cells *in vivo* and that it is this induction of TRAIL that is required for the IFN- $\gamma$ -mediated prevention of the formation of primary experimental tumours and experimental metastasis.<sup>15</sup>

A subpopulation of NK cells in adult mouse liver was shown to express TRAIL constitutively as a result of the autocrine production of IFN- $\gamma$ .<sup>14</sup> During development, TRAIL is predominantly expressed in fetal and neonatal mouse liver NK cells. Some of the TRAIL<sup>+</sup> immature NK cells remain in the liver of adult mice and its retention is dependent on IFN- $\gamma$ , but not on interleukin (IL)-12, IL-18 or host pathogens.<sup>16</sup> TRAIL could also be detected on IFN- $\gamma$ -producing killer dendritic cells (IKDCs), an important finding that provides an intriguing link between the innate immune system and the adaptive immune system.<sup>17,18</sup>

Expression of TRAIL at the mRNA level was shown in human peripheral blood lymphocytes following activation with a monoclonal anti-CD3 or phorbol 12-myristate 13-acetate (PMA)/ionomycin.<sup>19</sup> Increased expression of TRAIL was detected on CD4<sup>+</sup> and CD8<sup>+</sup> human peripheral blood T cells after T-cell receptor (TCR) stimulation, in combination with type I IFNs.<sup>20</sup> In contrast to CD95L, surface expression of TRAIL was not strongly induced by stimulation with TCR/CD3 alone.<sup>21</sup> The enhancement of TRAIL expression depended on the costimulation with interferon- $\alpha$  (IFN- $\alpha$ ) or IFN- $\beta$ . Ehrlich *et al.* reported that IFN- $\beta$  did not induce TRAIL on phytohaemagglutinin (PHA)- and IL-2-stimulated T cells.<sup>9</sup> This difference could be attributed to the different culture conditions

and to the activation status of the T cells. LPS, in combination with PHA and IL-2, also led to the up-regulation of TRAIL in a type I IFN-dependent manner.<sup>9</sup> These results indicate that type I IFNs can regulate TRAIL-mediated T-cell cytotoxicity. However, the question as to which cells may be the target of TRAIL expression remained elusive for quite some time. We will return to this issue later.

TRAIL and CD95L have also been implicated in the regulation of T helper 1 (Th1) and T helper 2 (Th2) responses (Fig. 2b). After stimulation with anti-CD3, *in vitro*-differentiated Th1 cells up-regulate CD95L, whereas Th2 cells express TRAIL. In addition, Th1 cells are more sensitive than Th2 cells to TRAIL-induced apoptosis, possibly because of the CD3-induced up-regulation of cellular FLICE inhibitory protein (c-FLIP) in Th2 cells.<sup>22,23</sup> Furthermore, inhibition of TRAIL (either by gene disruption or by RNA interference) in mice with allergic airway disease inhibited the production of the chemokine CCL20 and the homing of DCs and Th2 cells to the airways.<sup>24</sup> As a result, fewer Th2 cytokines were released and inflammation was reduced in TRAIL-deficient mice.

TRAIL has also been proposed to play a role in the regulation of haematopoiesis. Haematopoietic progenitor cells (CD34<sup>+</sup> cells) do not express TRAIL receptors and are therefore protected against TRAIL-induced apoptosis.<sup>25</sup> Mature erythroblasts are also resistant to TRAIL-induced apoptosis, in contrast to immature erythroblasts.<sup>26</sup> In patients with aplastic anaemia, TRAIL expression in the bone marrow was increased<sup>27</sup>, which could cause the death of immature erythroblasts. Furthermore, enhanced release of TRAIL was reported in Fanconi anaemia<sup>28</sup> and myelodysplastic syndrome.<sup>29</sup> By contrast, in patients with multiple myeloma, erythropoiesis is stimulated by the decreased expression of TRAIL-R1, TRAIL-R2 and TRAIL.<sup>30</sup> Furthermore, TRAIL was shown to affect peripheral blood-derived monocytes and granulocytes. However, this function was not mediated via the capacity of TRAIL to induce apoptosis, but rather by affecting adhesion of these cells to endothelial cells in response to inflammatory cytokines.<sup>31</sup>

In another study, the TRAIL/TRAIL-R system was shown to play a decisive role in the homeostasis of a particular subset of CD8<sup>+</sup> T cells. So-called 'helpless' CD8<sup>+</sup> T cells that are primed in the absence of CD4<sup>+</sup> T cells – and thus in the absence of help – are unable to undergo a second round of clonal expansion upon restimulation with their cognate antigen.<sup>32</sup> As TRAIL-deficient 'helpless' CD8<sup>+</sup> T cells could still expand a second time, this effect is thought to be mediated via TRAIL. Therefore, a mechanism was suggested in which non-helped T cells are eliminated via TRAIL by an activation-dependent killing upon antigen rechallenge.<sup>33</sup> More recently, IL-15 was identified to be a mediator of this effect by rendering 'helped' CD8<sup>+</sup> T cells resistant to TRAIL-induced apoptosis (Fig. 2a).<sup>34</sup>

In a publication that makes reference to historic, highly contested results on so-called 'suppressor' T cells that were first described over 30 years ago,<sup>35–37</sup> an immunosuppressive function of TRAIL was reported.<sup>38</sup> In the original publications dealing with suppressor T cells, the factor responsible for the observed suppression effect remained elusive. By performing some of the original experiments that were used to study suppressor T cells, but now employed TRAIL<sup>−/−</sup> mice and recombinant TRAIL, Griffith *et al.*<sup>38</sup> provides quite compelling evidence that TRAIL may be the long thought-after suppressor factor.

## TRAIL and autoimmunity

The TRAIL/TRAIL receptor system also seems to be implicated in a variety of autoimmune diseases, or at least capable of modulating an autoimmune response. TRAIL<sup>−/−</sup> mice and TRAIL-R<sup>−/−</sup> mice do not show signs of spontaneous autoimmunity; however, recombinant TRAIL was able to inhibit autoimmune diseases in a number of animal models. A summary of phenotypes of TRAIL<sup>−/−</sup> and TRAIL-R<sup>−/−</sup> mice, referring to the function of the TRAIL/TRAIL-R system in immunology, is shown in Table 1.

In a model of collagen-induced arthritis, blockage of TRAIL increased the severity of the disease, whereas gene transfer of TRAIL prevented it.<sup>3</sup> In another report it was shown that collagen-induced arthritis was suppressed using DCs pulsed with collagen and an inducible adenoviral TRAIL expression construct.<sup>48</sup> In mice that received collagen-treated TRAIL-expressing DCs, the extent of joint inflammation was significantly lower than in the control group. In these mice, T-cell proliferation and IFN- $\gamma$  production was decreased, resulting in the suppression of arthritis. In rheumatoid arthritis, which is characterized by the expansion of fibroblast-like synoviocytes (FLSs), a dual role for TRAIL was suggested. It was shown that TRAIL can induce apoptosis as well as the proliferation of FLSs.<sup>49</sup>

Moreover, TRAIL<sup>−/−</sup> mice were more susceptible to autoimmune arthritis and diabetes.<sup>42,50</sup> Diabetes development in non-obese diabetic (NOD) mice was strongly enhanced in TRAIL<sup>−/−</sup> mice after induction with cyclophosphamide or streptozotocin.<sup>42</sup> These models are similar to human type I diabetes and require the participation of macrophages and T cells. Furthermore, blockage of TRAIL with a fusion protein of the extracellular domain of TRAIL-R2 containing the Fc portion of human IgG1 (TRAIL-R2-Fc) led to enhanced type I diabetes in NOD mice.<sup>51</sup> It is, however, unclear which cells are responsible for this autoimmunity-preventing effect of TRAIL and which cells are affected by it. Knowledge of this may help in designing rational approaches to prevent the deletion of  $\beta$ -islet cells.

Phenotypes (TRAIL <sup>-/-</sup> mice)	References
Partial resistance to <i>Listeria monocytogenes</i> infection	39
'Helpless' CD8 <sup>+</sup> T cells can proliferate upon secondary stimulation	33
Prolonged memory function of 'helpless' CD8 <sup>+</sup> T cells	40
Lack of tolerance induction by 'helpless' CD8 <sup>+</sup> T cells to apoptotic cells	38
Increased symptoms of experimental bacterial meningitis	41
Reduced allergic airway disease with reduced inflammation	24
Defects in negative selection, increased susceptibility to autoimmune diseases: collagen-induced arthritis, streptozotocin-induced diabetes	42
Normal negative selection, no sign of spontaneous autoimmunity	43,44
Increased severity of EAE in remitting disease and in non-remitting disease	45
Increased clearance of MCMV, increased levels of IL-12, IFN- $\alpha$ and IFN- $\gamma$ , enhanced macrophage cytokine production	46
Reduced tissue apoptosis after radiation in thymus, spleen, Peyer's patches and white matter of the brain	47

**Table 1.** Immunological phenotypes of TRAIL<sup>-/-</sup> and TRAIL-R<sup>-/-</sup> mice

Intriguingly, in experimental autoimmune encephalomyelitis (EAE), which is widely used as an animal model for multiple sclerosis (MS), TRAIL blockage led to a higher degree of inflammation in the central nervous system (CNS) and a more severe disease, which was also confirmed by studies using TRAIL<sup>-/-</sup> mice. However, the degree of apoptosis of inflammatory cells in the CNS was not affected by the blockage of TRAIL, suggesting that TRAIL does not regulate apoptosis of inflammatory cells but prevents the activation of autoreactive T cells.<sup>45,52</sup> In another study, the exact opposite effect of TRAIL blockage was observed when TRAIL-blocking TRAIL-R2-Fc was injected into the CNS. The clinical severity of EAE, as well as the neural apoptosis in brainstem motor areas, was significantly reduced. This was a result of less TRAIL-induced apoptosis of neuronal cells by TRAIL-expressing encephalitogenic T cells.<sup>53</sup> Therefore, TRAIL not only has an immunoregulatory role in the periphery, but also contributes to neural damage in the inflamed brain.

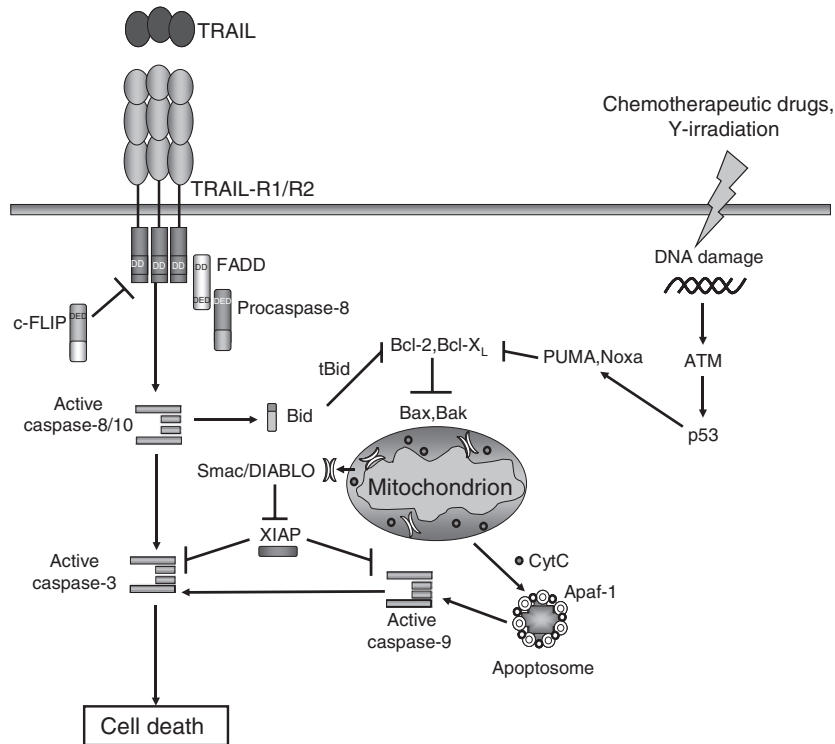
TRAIL was also implicated in experimental autoimmune thyroiditis. Treatment with recombinant TRAIL led to a milder form of the disease with a significant decrease in mononuclear cell infiltration in the thyroid and less thyroid follicular destruction.<sup>54</sup> In addition, apoptosis of thyrocytes and thyroglobulin-specific Th1 responses were decreased as a result of less IFN- $\gamma$  production. In this model, the presence of TRAIL led to less IFN- $\gamma$ , which, in turn, regulated the production of chemokines. Thereby the infiltration of mononuclear cells and lymphocytes into the thyroid gland was reduced. These findings are in line with reports that TRAIL-R<sup>-/-</sup> mice show elevated levels of IFN- $\gamma$ <sup>46</sup> and the previously mentioned role of TRAIL in the Th1/Th2 balance.<sup>22-24</sup>

The influence of TRAIL on autoimmunity was at first attributed to its supposed role in thymic negative selection. However, a function of the TRAIL/TRAIL-R system in central tolerance is, to say the least, highly contested. Albeit an initial study showed that negative selection of

human and mouse thymocytes is independent of TRAIL signalling,<sup>55</sup> two reports that contradicted the previous study suggested that TRAIL was necessary for intrathymic selection.<sup>50,56</sup> However, TRAIL expression has not been detected on thymic dendritic and epithelial cells, which are the most important mediators of negative selection in the thymus.<sup>57,58</sup> Finally, elegant studies in various different model systems for the study of negative selection by Cretney *et al.*,<sup>43</sup> using TRAIL<sup>-/-</sup> mice and a neutralizing anti-mouse TRAIL monoclonal antibody, confirmed and genetically proved that negative selection in the thymus did not involve the TRAIL system. Additionally, negative selection was also reported to be normal in TRAIL-R<sup>-/-</sup> mice.<sup>46</sup> In conclusion, it is rather unlikely that the TRAIL/TRAIL-R system plays a role in thymic negative selection under physiological conditions.

### TRAIL in viral and bacterial infections

Many viruses are able to induce immunosuppression. However, the mechanisms of how viruses inhibit cell-mediated immunity are not completely understood. One possible mechanism was introduced by Vidalain *et al.*<sup>59</sup> Those authors showed that infection with measles virus led to TRAIL-mediated killing of activated T cells by monocyte-derived DCs, thereby down-regulating antiviral immune responses. Moreover, the release of cytotoxic factors from DCs was reported after infection with human immunodeficiency virus type I (HIV-1).<sup>60</sup> In this study it was suggested that CD95L and TNF- $\alpha$  participate in the induction of uninfected thymocyte killing by HIV-1-infected DCs. However, the involvement of TRAIL was not investigated. Recently it was reported that HIV-1 infection causes the production of type I IFNs by plasmacytoid DCs, which in turn leads to the expression of membrane-bound TRAIL on CD4<sup>+</sup> T cells and TRAIL production by monocytes. In addition, binding of HIV-1 to CD4<sup>+</sup> T cells up-regulates TRAIL-R2, and this has been



**Figure 1.** Schematic overview of the human tumour necrosis factor-related apoptosis-inducing ligand (TRAIL) apoptosis pathway. Binding of TRAIL to TRAIL-R1 and/or TRAIL-R2 leads to receptor trimerisation and formation of the death-inducing signalling complex (DISC). The adaptor protein Fas-associated death domain (FADD) is recruited to the DISC where the death domains (DD) of both proteins interact. Subsequently, pro-caspases 8 and 10 are recruited to the DISC where they interact with FADD via their death effector domains (DEDs). DISC-activated caspases 8 and 10 then trigger a caspase cascade by cleavage of caspase-3. In addition, Bid is cleaved into tBid, which initiates the mitochondrial apoptosis pathway leading to release of cytochrome *c* (CytC) and second mitochondria derived activator of caspases/direct IAP binding protein with low pI (Smac/DIABLO) from the mitochondria. CytC, together with apoptotic protease activating factor 1 (Apaf-1) forms the apoptosome, an activation platform for caspase-9. Smac/Diablo counteracts the inhibitory function of X-linked inhibitor of apoptosis protein (XIAP), thereby allowing for full activation of caspases 3 and 9, ultimately leading to cell death. The intrinsic, Bcl-2-controlled, pathway is also triggered after extensive DNA damage. BH3-only proteins PUMA and Noxa are up-regulated, thereby altering the ratio of pro-apoptotic and anti-apoptotic Bcl-2-family members, allowing for mitochondrial depolarization and cell death. c-FLIP, cellular FLICE inhibitory protein.

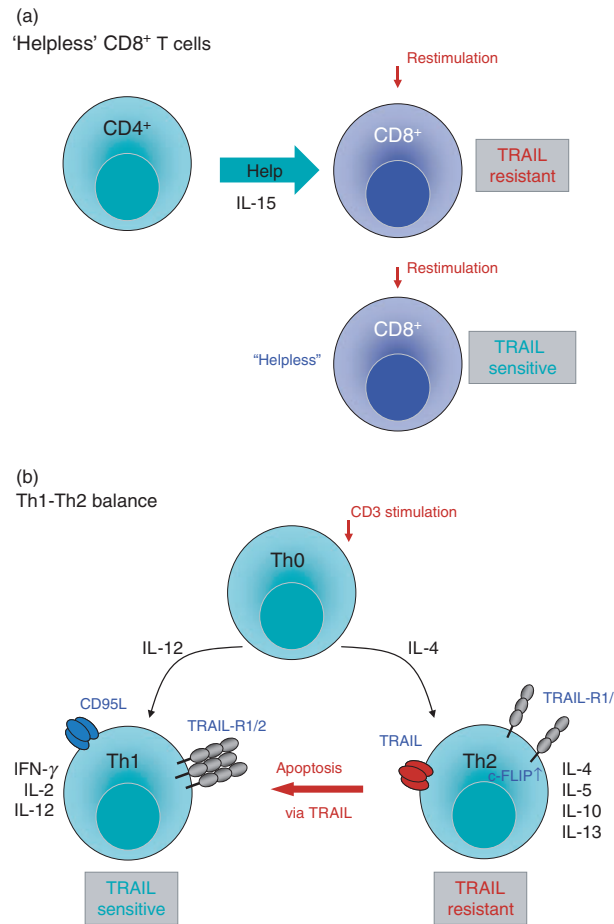
suggested to facilitate the selective apoptosis of CD4<sup>+</sup> T cells.<sup>61–65</sup>

Sedger *et al.* demonstrated that TRAIL-resistant fibroblasts could be sensitized to TRAIL-induced apoptosis by infection with human cytomegalovirus (HCMV).<sup>66</sup> The infection caused the up-regulation of TRAIL-R1 and TRAIL-R2 on infected fibroblasts, whereas IFN- $\gamma$ , which is produced by T and B lymphocytes, NK cells, monocytes and macrophages, induced expression of TRAIL and down-regulated the expression of TRAIL-Rs on uninfected fibroblasts. Hence, TRAIL selectively kills virus-infected cells while leaving uninfected cells intact.

An antiviral response against encephalomyocarditis virus (ECMV) that was mediated by TRAIL-expressing NK cells was shown to be dependent on IFN- $\alpha$  and IFN- $\beta$ , which are produced by virus-infected cells. Blocking of NK cell-derived TRAIL resulted in higher viral titres and earlier death of infected mice.<sup>67</sup>

TRAIL was also implicated in chronic hepatitis C virus (HCV) infection. Under normal conditions, CD1c-expressing myeloid DCs kill their target cells mainly via TRAIL. This ability was completely abolished in patients with chronic hepatitis C.<sup>68</sup> However, TRAIL-induced apoptosis in the viral defence against HCV also occurs via cytotoxic T cells (Fig. 3). CD8<sup>+</sup> T cells in HCV-infected liver express CD95L<sup>69</sup> and TRAIL<sup>70</sup> and can induce the apoptosis of infected hepatocytes,<sup>71,72</sup> whereas normal hepatocytes are resistant to TRAIL.<sup>73</sup> Furthermore, HCV infection has been shown to sensitize human hepatocytes to TRAIL-induced apoptosis.<sup>74</sup>

During primary infection with influenza virus, elimination of the virus-infected cells is thought to be controlled by cytotoxic T cells through perforin-mediated and CD95L-mediated mechanisms.<sup>75,76</sup> However, recent studies suggest that CD8<sup>+</sup> T cells can also kill influenza virus-infected cells via TRAIL.<sup>77</sup> Using TRAIL<sup>-/-</sup> mice, Brincks *et al.*<sup>78</sup> showed that TRAIL



**Figure 2.** Tumour necrosis factor-related apoptosis-inducing ligand (TRAIL) in T-cell homeostasis and differentiation. (a) So-called 'helpless' CD8<sup>+</sup> T cells that are primed in the absence of CD4<sup>+</sup> T-cell help are unable to undergo a second round of clonal expansion upon restimulation with their cognate antigen because they are eliminated by activation-dependent killing via TRAIL.<sup>30,31</sup> By contrast, 'helped' CD8<sup>+</sup> T cells are resistant to TRAIL-induced apoptosis, probably as a result of IL-15.<sup>32</sup> (b) After stimulation with anti-CD3, T helper 0 (Th0) cells can differentiate into T helper 1 (Th1) cells in the presence of interleukin (IL)-12 or into T helper 2 (Th2) cells in the presence of IL-4. Th1 cells express CD95L, whereas Th2 cells up-regulate TRAIL on their surface. Th1 cells can then be killed by TRAIL, whereas Th2 cells are resistant to TRAIL-induced apoptosis, possibly as a result of the up-regulation of cellular FLICE inhibitory protein (c-FLIP) in Th2 cells.<sup>22,23</sup>

deficiency leads to increased titres of influenza virus and increased disease severity. Adoptive transfer of TRAIL<sup>+/+</sup> effector CD8<sup>+</sup> T cells rescued mice that were injected with a lethal dose of influenza virus, while CD8<sup>+</sup> T cells from TRAIL<sup>-/-</sup> mice could not save those mice. However, the migration to the lungs was not altered for TRAIL<sup>+/+</sup> and TRAIL<sup>-/-</sup> effector T cells. Concluding these data, TRAIL deficiency leads to a more severe influenza infection by decreasing CD8<sup>+</sup> T cell-mediated cytotoxicity.

Strong up-regulation of TRAIL, TRAIL-R1 and TRAIL-R2 in response to respiratory syncytial virus (RSV) was observed in primary tracheal-bronchial cells, A549 and HEp-2 cells.<sup>79</sup> In addition, RSV-infected cells became highly sensitive to TRAIL-induced apoptosis. These results suggest that RSV-infected cells could be eliminated by TRAIL-expressing immune cells *in vivo*.

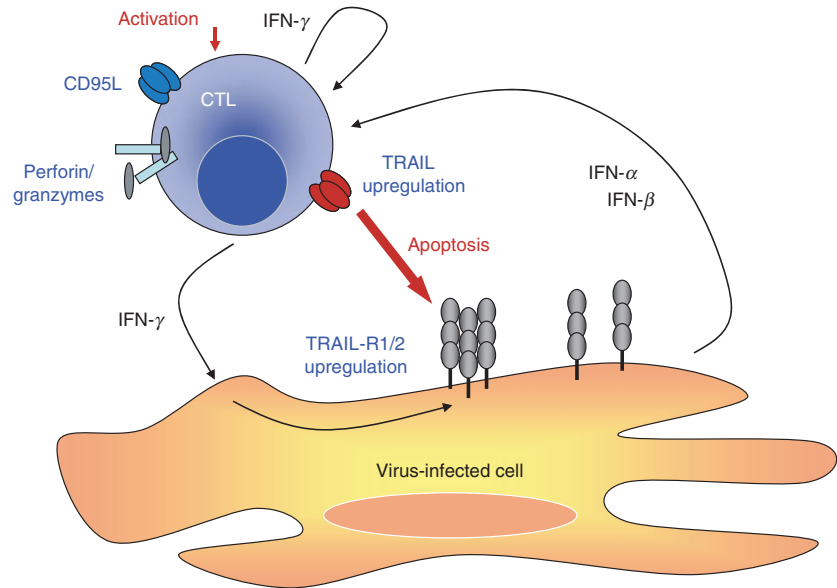
A study by Diehl *et al.* investigated the effect of a variety of different pathogens in TRAIL-R<sup>-/-</sup> mice.<sup>46</sup> TRAIL-R<sup>-/-</sup> mice have an apparently normal lymphocyte make-up but, interestingly, they display enhanced innate immune responses when challenged with certain infectious agents. The authors compared the response of TRAIL-R<sup>-/-</sup> mice with wild-type control mice when infected with *Listeria monocytogenes*, *Salmonella typhimurium*, *Mycobacterium bovis*, bacille Calmette-Guérin (BCG), ECMV and murine cytomegalovirus (MCMV).

TRAIL up-regulation and enhanced cytokine production was observed in TRAIL-R<sup>-/-</sup> cells after stimulation of macrophages with *M. bovis*. However, the strongest difference between TRAIL-R<sup>+/+</sup> and TRAIL-R<sup>-/-</sup> mice was the enhanced resistance of TRAIL-R<sup>-/-</sup> mice against MCMV. In TRAIL-R<sup>-/-</sup> mice, the increased clearance of MCMV correlated with increased levels of IL-12, IFN- $\alpha$  and IFN- $\gamma$  that are presumably produced by DCs, macrophages and NK cells. Thus, TRAIL might negatively regulate innate immune responses by influencing antigen-presenting cells.

Taken together, these results suggest that the TRAIL/TRAIL-R system has developed as a receptor-ligand system specifically geared to fight infections and to control immune responses. It is tempting to speculate that differences in the infection challenges between different mammalian species may have caused the surprisingly divergent evolution of TRAIL-R in humans compared with mice and other species.

## TRAIL and tumour immunity

The importance of the immune system in controlling tumour growth is now well established.<sup>80-82</sup> Exogenous TRAIL was shown to kill tumour cells selectively without negatively affecting normal cells, making TRAIL and TRAIL-receptor agonists promising anticancer therapeutics.<sup>83,84</sup> A first indication that endogenous TRAIL may suppress tumour growth came when Sedger *et al.*<sup>66</sup> reported that a syngeneic tumour transplant of a B-cell lymphoma line displayed enhanced tumour growth in TRAIL<sup>-/-</sup> mice. Moreover, endogenous TRAIL on NK cells that were stimulated with IFN- $\gamma$  or IL-12 was able to kill even disseminated tumour cells effectively in the liver but not in the lung after implantation of metastasising breast and renal carcinoma cells.<sup>15,85,86</sup> Furthermore, NK T cells stimulated by  $\alpha$ -galactosylceramide ( $\alpha$ -GalCer) showed an effective TRAIL-mediated anti-tumour effect.<sup>14,87</sup>



**Figure 3.** Tumour necrosis factor-related apoptosis-inducing ligand (TRAIL)-mediated killing of virally infected cells depends on type I and II interferons (IFNs). Virus infection leads to upregulation of TRAIL-R1 and TRAIL-R2 on the surface of the infected cell. In addition, IFN- $\alpha$  and IFN- $\beta$  are produced. These type I IFNs, together with IFN- $\gamma$ , which is autocrinely produced by activated CD8<sup>+</sup> cytotoxic lymphocytes (CTLs) cause the upregulation of TRAIL on CTLs, which can, in turn, kill the virus-infected cell via TRAIL.<sup>66–70</sup>

While TRAIL suppressed the growth of injected tumour cell lines and experimental metastasis of TRAIL-sensitive cell lines, the role of TRAIL-mediated tumour surveillance, especially against autochthonous tumours, is rather unclear. Lymphomas and carcinogen-induced tumours grow faster in the absence of TRAIL or TRAIL-R.<sup>87–89</sup> For example, E $\mu$ -myc-induced lymphomas, lymphomas in aged mice and lymphomas induced by heterozygous loss of p53 were increased in the absence of TRAIL or TRAIL-R.<sup>83,84</sup> However, intestinal tumours and mammary carcinomas were not affected.<sup>89,90</sup>

The first indication of a metastasis-specific surveillance function of TRAIL was shown in an autochthonous multi-step model of skin tumorigenesis. When TRAIL-R<sup>-/-</sup> mice were treated with the tumour-initiating and tumour-promoting agents 9,10-dimethylbenz-A-anthracene (DMBA) and 12-O-tetradecanoylphorbol-13-acetate (TPA), papillomas and carcinomas developed without the influence of the TRAIL/TRAIL-R system. Surprisingly, lymph node metastases were greatly enhanced in the absence of TRAIL-R, which was explained by the fact that tumour cells gained TRAIL sensitivity by loss of adhesion.<sup>91</sup> However, whether this specific metastasis suppressor function of TRAIL-R is confined to metastases in lymphoid organs, and which cells are responsible for the TRAIL-mediated effect, is still under investigation. More importantly, it needs to be determined if a role of TRAIL in tumour and/or metastasis surveillance has implications for the therapeutic use of TRAIL-R agonists against primary tumours or as an adjuvant therapy against metastasising tumours.

The potential impact of TRAIL-R agonists in anti-tumour immune therapy was demonstrated by a sophisticated approach using three different mAbs. This so-called 'TrimAb' therapy comprised an activating anti-

body to TRAIL-R that induced an immunogenic death of tumour cells, a DC stimulating antibody to CD40 and an activating antibody to 4-1BB that provided co-stimulation to cytotoxic T lymphocytes (CTLs).<sup>92</sup> Combination of these three antibodies led to the rejection of established tumours, which required the presence of CD8<sup>+</sup> T cells and IFN- $\gamma$ . Intriguingly, even co-established TRAIL-resistant tumours were rejected with the 'TrimAb' therapy when only 10% TRAIL-sensitive tumour cells were present. This effect was mainly caused by the recruitment of Fc receptor-expressing cells to cells killed by the activating antibody to TRAIL-R, MD5.1, most importantly macrophages and DCs, which, in turn, activated CTLs and induced TRAIL-independent tumour cell death.<sup>93</sup>

### Concluding remarks

So far, TRAIL has been shown to exert immunosuppressive and immunoregulatory functions important for T-cell homeostasis and for the innate-to-adaptive immunity transition. Furthermore, TRAIL plays a role in the clearance of certain viral infections and in the immune surveillance against tumours and metastasis.

Hence, the studies concluded so far could already unravel a number of secrets concerning the role(s) of TRAIL and its receptors in the immune system. However, to understand the importance of this receptor–ligand system and its intricate regulation at the cellular and molecular levels, it will be necessary to identify the specific effector and target cells in the various systems. This can be achieved by performing studies that build on the results summarized in this review and carried out in mice in which TRAIL and TRAIL-R are individually targeted in defined cellular subpopulations.

## Disclosures

The authors have no conflict of interest.

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